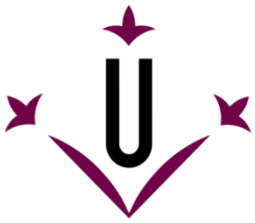

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Encapsulation and stability of a phenolic-rich extract from mango peel within water-in-oil-in-water emulsions

Gustavo R. Velderrain-Rodríguez[a], Alejandra Acevedo-Fani[b], Gustavo A. González-Aguilar[a], Olga Martín-Belloso O[b]*

[a] Coordinación de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en Alimentación y Desarrollo, A.C. Carretera a la Victoria Km 0.6. C.P. 83304, Hermosillo, Sonora, México.

[b] Department of Food Technology, University of Lleida – Agrotecnio Centre, Av. Alcalde Rovira Roure 191, 25198, Lleida, Spain

*Corresponding author: Professor Olga Martín-Belloso. Tel.: +34 973702593. e-mail: omartin@tecal.udl.es

Abstract

Mango peel is an excellent source of compounds with nutritional and functional properties, especially phenolic compounds (PC). However, it is usually discarded due to its unpleasant taste and its difficulty to be added to food products. As a solution, we evaluated the feasibility of encapsulating a phenolic-rich extract from mango peel (MPPE) within water-in-oil-in-water ($W_1/O/W_2$) emulsions, using different surfactants (Tween 20, Tween 80 and lecithin). Time and amplitude conditions of ultrasound treatment were evaluated to form the MPPE loaded water-in-oil (W/O) emulsions, using polyglycerol polyricinoleate (PGPR) as a surfactant. Regarding of $W_1/O/W_2$ emulsions, the highest encapsulation efficiency (EE) was observed in those with Tween 20 and lecithin (98.65 ± 1.14% and 96.11 ± 1.37%, respectively). However, emulsions with Tween 80 had the best physical and encapsulation stability (ES) during storage. These results demonstrate that PC can be successfully encapsulated within efficient and stable emulsion-based systems by selecting the appropriate surfactants.

Keywords.

Polyphenols; Mango by-products; Encapsulation; Surfactant; Double emulsions; Emulsification

1. Introduction

Mango peel is a non-edible fruit part usually discarded, even though it has the higher phenolic compounds (PC) content compared to its pulp or seed. It is a rich source of water-soluble phenolic acids (PA), being gallic acid the most ubiquitous and abundant compound (commonly found as a part of gallotannins) (Sáyago-Ayerdi et al., 2013; Velderrain-Rodríguez, G. et al., 2015). Recent studies have shown that among the other PC found in mango (cv. 'Ataulfo') peel, gallic acid has the highest intestinal permeability in a CaCo-2/HT29 monolayer model, and it also has the highest antiproliferative activity against LS180 human colon cancer cells, which suggest that its addition PC into functional food products may promote intestinal health (Pacheco-Ordaz, Antunes-Ricardo, Gutiérrez-Uribe, & González-Aguilar, 2018; Velderrain-Rodríguez, G. R. et al., 2018).

Currently, there are not enough data to establish dietary intake recommendations for PC. Following the 5-a-day (≈ 400-500 g portion of fruits) intake, suggested by international health agencies (such as the World Health Organization), the total PC intake goes around 800-1000 mg, as discussed by Williamson and Holst (2008). In that sense, mango peel represents a higher source of PC, as it contains around 100 mg per gram of raw mango peel (Ajila, Aalami, Leelavathi, & Rao, 2010; Okino Delgado & Fleuri, 2016). However, as it has an unpleasant taste, recent studies have been aiming at the incorporation of mango peel within the formulation of novel functional food products, such as bakery products or healthy snacks (Ajila et al., 2010; Blancas-Benitez, de Jesús Avena-Bustillos, Montalvo-González, Sáyago-Ayerdi, & H. McHugh, 2015; Ramírez-Maganda et al., 2015).

Nevertheless, different studies suggest that PA are prone to oxidation during traditional food processing conditions (light exposure, high temperature, pH changes) affecting their beneficial properties (Krook & Hagerman, 2012; Rothwell et al., 2015; Velderrain-Rodriguez et al., 2016). Commonly, these losses are related to the deprotonation of PC hydroxyl moieties at pH above their pKa values (usually around 4.5–4.9)(Miyague, Macedo, Meca, Holley, & Luciano, 2015), or thermal decarboxylation after using high temperature treatments (Kaur, Whitson,

Ashton, Katopo, & Kasapis, 2018). Moreover, Kaur et al. (2018) reported that storage at 40 °C leads to a significant reduction of the PA content in oat samples of industrial interest. However, as either domestic or industrial food processing causes significant losses of PC in common food products (Rothwell et al., 2015), encapsulation methods should be used to provide protection prior its addition (Ribas-Agustí, Martín-Belloso, Soliva-Fortuny, & Elez-Martínez, 2018).

Emulsion-based encapsulation systems are gaining popularity as an alternative solution to overcome these problems, as they can be applied in functional food products to incorporate either, or both, hydrophilic and hydrophobic bioactive compounds (Aditya et al., 2015). Among emulsion-based systems, double emulsions represent a healthier and promising strategy to incorporate PC, as their use may reduce fat content in food products such as salad dressings and cheese (Gharehbeglou, Jafari, Hamishekar, Homayouni, & Mirzaei, 2019). Moreover, double emulsions may improve the stability of PC during storage as they reduce interactions with other food components (such as proteins and polysaccharides) or their exposure to environmental factors, (such as temperature, light, moisture, and oxygen). Recently, de Almeida Paula et al. (2018) reported that the use of double emulsions increased the half-life of anthocyanins by 2.4-fold, with an improved thermal and color stability for 10 days at 40 °C in the presence of light.

Double emulsions are systems in which an inner emulsion is dispersed as fine droplets within another continuous phase, and stabilized using a second surfactant. According to their composition double emulsions are usually categorized as oil-in-water-in-oil ($O_1/W/O_2$) or water-in-oil-in-water ($W_1/O/W_2$). In that sense, $W_1/O/W_2$ emulsions can protect water-soluble bioactive compounds, loaded in the innermost compartment of emulsions, from oxidation or other detrimental effects. In $W_1/O/W_2$ emulsions, inner water-in-oil (W_1/O) emulsions must be formed first and stabilized using hydrophobic surfactants. Small sizes of the W_1/O droplets reduces the effect of gravity and Brownian motion and may become more stable as primary inner emulsions (Guerra-Rosas, Morales-Castro, Ochoa-Martínez, Salvia-Trujillo, & Martín-Belloso, 2016). Subsequently, the W_1/O emulsion must be dispersed as

droplets in a continuous second aqueous phase (W_2), commonly using less shear stress to prevent the disruption of W_1/O primary emulsions, and using the appropriate hydrophilic surfactants to stabilize them (Faridi Esfanjani, Jafari, & Assadpour, 2017).

Surfactants are usually selected according to their Hydrophilic-Lipophilic Balance (HLB), being those with higher values (above 11.0) considered as hydrophilic surfactants and those with lower values (below 9.0) as lipophilic surfactants (Macedo et al., 2006). The surfactant selection depends on the type of emulsion intended. Lipophilic surfactants are usually used to produce water-in-oil emulsions and hydrophilic surfactants for oil-in-water emulsions. Polyglycerol polyricinoleate (PGPR) is the most common lipophilic surfactant used for W_1/O emulsion due to its effectiveness and stability (Andrade & Corredig, 2016). Conversely, among hydrophilic surfactants, Tween (polysorbate) and Span (sorbitan) series are the most used. As surfactants adsorb to the interface, these molecules lower emulsions' interfacial tension, keeping a smooth surface and preventing coalescence of droplets (Jiang et al., 2018). Thus, the HLB value of surfactants, coupled with shear stress conditions, needs to be carefully selected to achieve long-term stable $W_1/O/W_2$ emulsion systems.

The encapsulation efficiency of double emulsions may vary according to the surfactant use and its HLB value. However, choosing an appropriate hydrophilic surfactant for $W_1/O/W_2$ emulsion purely based on its HLB value is not that simple, as part of the lipophilic surfactant migrates to the external O/W interface and influence the formation of multiple droplets (Zhang, Zhai, Ou, Song, & Zhang, 2018). Schmidts, Dobler, Nissing, and Runkel (2009) reported that the chemical structure and compatibility of Tween 20 and 80 are linked to the emulsions encapsulation stability, as they observed better stability in emulsions using Tween 80 when combined with span 80 as the lipophilic surfactant. Even though Tween 20 and Tween 80 possess a similar HLB value, they have different chemical structures that may enhance interfacial interactions with the lipophilic surfactant.

Tween 20 has saturated laurate-chains with only 12 carbon atoms, whereas Tween 80 has an oleate-chain with 18 carbon atoms and one unsaturated bond.

Data regarding the effect of the hydrophilic surfactants selection in double emulsions formulation is still scarce. In addition, some studies reported that the stability of double emulsions might be affected by migration of encapsulated PC from W_1 to the oil phase or W_2, due to their amphiphilic properties. Recently, Katsouli, Polychniatou, and Tzia (2017) reported that PA from olive oil have surface-active properties, and its addition to water-in-oil emulsions can modify their oxidative stability, particle size, and turbidity. In that sense, the role hydrophilic surfactants on $W_1/O/W_2$ emulsions' stability along storage may vary according to the different species among the encapsulated PC extract. Thus, the objective of this study was to evaluate the stability of a mango peel phenolic-rich extract (MPPE) encapsulated within $W_1/O/W_2$ emulsions, and the role of surfactants on the emulsions' physical stability and encapsulation properties (encapsulation efficiency and stability) during storage conditions at 4 °C.

2. Materials and methods

2.1. Materials

Mangoes (*M. indica* L., cv. Ataulfo) at ripening stage IV were selected according to the characteristics described by Palafox-Carlos et al. (2012), and purchased from a local market in Hermosillo, Sonora, Mexico. Polysorbate 20 (Tween 20; HLB 16.7), Polysorbate 80 (Tween 80; HLB 15), powder soy lecithin (HLB 8) and all solvents were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Glycerol was provided from Fischer Scientific (UK). Polyglycerol polyricinoleate (PGPR) was provided by Danisco (Denmark). Corn oil was purchased from a local market in Lleida, Spain, whereas sunflower oil was provided by Aceites Borges Pont, S.A.

2.2. Mango peel phenolic-rich extract (MPPE)

Freeze-dried and powdered peel from mango cv. 'Ataulfo' was used for PC extraction. The PC were extracted from mango peel by alkaline hydrolysis to release those compounds that were strongly associated with the food matrix, as

described by Mattila and Kumpulainen (2002), with minor modifications as suggested by Velderrain-Rodríguez, G. R. et al. (2018). The extracted fraction of PC was dried overnight at room temperature to finally obtain the desired MPPE powder. The MPPE used in this study has been previously characterized by Velderrain-Rodríguez, G. R. et al. (2018). These authors reported that the most abundant phenolic compound within MPPE is gallic acid (23.81 ± 2.84 mg/g), followed by mangiferin (0.96 ± 0.02 mg/g) and 2-Hydroxybenzoic acid (0.70 ± 0.00 mg/g), and the flavonoids catechin (0.06 ± 0.00 mg/g) and quercetin (0.03 ± 0.00 mg/g).

Previous to its encapsulation within double emulsion systems, MPPE (1 mg/mL) was dissolved in a 0.1 M NaCl solution (aqueous phase) to induce a balance between Laplace and osmotic pressures of emulsions. In addition, the MPPE-NaCl solution was filtered (at the day of use) through a 0.22 µm nylon membrane filter prior to its encapsulation. The total amount of PC dissolved in this aqueous phase was quantified by the Folin-Ciocalteu method, as described by Mazzucotelli et al. (2017), and expressed as mg of gallic acid equivalents (GAE).

2.3. Double emulsion system

Emulsions were obtained according to the method described by Aditya et al. (2015), with minor modifications. Usually, preparing double emulsion systems requires a two-stage process: 1) Inner water-in-oil (W_1/O) emulsions preparation by W_1 droplets dispersion in the oil phase; 2) Water-in-oil-in-water (W_1/O/W_2) emulsion systems preparation, by the dispersion of the inner W_1/O emulsions in a second water phase (W_2).

2.3.1. Inner W_1/O emulsion

The inner MPPE W_1/O emulsions were prepared using the previously described MPPE (dissolved in 0.1 M NaCl solution), whereas control W_1/O emulsions used a pure NaCl (0.1 M) solution as inner aqueous phase (W_1). The W_1/O emulsions consisted of 70% (w/w) corn oil, 22% (w/w) aqueous phase, 5% (w/w) PGPR as surfactant and 3% (w/w) glycerol as cosurfactant. Before emulsification, PGPR was

dissolved in corn oil, whereas glycerol was dissolved in W_1 under magnetic stirring at 60 °C for 5 min. The dispersion of W_1 in corn oil was performed using an Ultra-Turrax T25 homogenizer at 6,000 rpm for 8 min. Subsequently, the mixture was treated with a UPS400S Hielscher sonifier (Hielscher Ultrasound Technology, Teltow, Germany), of 400 W nominal power and a frequency of 24 kHz equipped with a 22-mm sonotrode. The particle size and span of W_1/O emulsions at different ultrasound amplitudes (40, 60, 80, 100 µm) and treatment time (1, 2, 3 min) were evaluated.

2.3.2. Preparation of $W_1/O/W_2$ emulsion

Double emulsions were prepared with 25% (w/w) of W_1/O and 75% (w/w) of a second aqueous phase (W_2), which was prepared using a Milli-Q water-NaCl (0.1M) solution and 2% (w/w) of one of the tested hydrophilic surfactants (either Tween 20, Tween 80 or lecithin) for $W_1/O/W_2$ emulsions. The addition of NaCl to aqueous inner and outer compartments of emulsions may induce a balance between Laplace and osmotic pressures, reducing collision frequency of water droplets by lowering attractive forces and decreasing the dielectric constant of the aqueous phases, (Momeny, Mirhosseini, & Sarker, 2017). The different hydrophilic surfactants were evaluated to select the most suitable for MPPE encapsulation in stable double emulsion systems. For $W_1/O/W_2$ preparation, W_1/O dispersion into W_2 was performed using an Ultra-Turrax T25 homogenizer at 3,000 rpm for 4 min. Likewise, the mixture was treated with a UPS400S Hielscher sonifier at 50 µm for 90 s. Aliquots of each $W_1/O/W_2$ emulsion were taken at 0, 1, 2, 3, 5, 7, 10, 15 and 21 days during storage at 4 °C for further analysis.

2.4. Droplet size and distribution

The particle size of W_1/O and $W_1/O/W_2$ was measured by static light scattering technique using a Mastersizer 3000 (Malvern Instruments Ltd, Worcestershire, UK). For W_1/O emulsions, sunflower oil was used as the continuous phase, whereas for $W_1/O/W_2$ distilled water was used, fixing a refractive index of 1.473 and 1.333, respectively. Data was reported as volume-weighted average ($d_{4,3}$) and

expressed in μm. The particle size distribution was expressed in terms of span, defined according to the following Equation (1):

$$Span = \frac{D(\nu, 0.9) - D(\nu, 0.1)}{D(\nu, 0.5)} \quad (1)$$

where $D(\nu, 0.9)$, $D(\nu, 0.5)$, $D(\nu, 0.1)$ correspond to diameters at which 90%, 50% and 10% of the particles' volume are of a smaller size, respectively. Thus, lower span values are related to more monodisperse distributions.

2.5. Emulsions viscosity

Aliquots of 10 mL of emulsion were used to measure the apparent viscosity of $W_1/O/W_2$ emulsions, as described by Artiga-Artigas, Acevedo-Fani, and Martín-Belloso (2017) using a vibro-viscometer (SV-10, A&D Company, Tokyo, Japan) vibrating at 30 Hz and constant amplitude (0.4 mm). Results were expressed as mPa•s and followed along storage at 4 °C.

2.6. Emulsion physical stability

The double emulsions physical stability was evaluated during 21 days of storage at 4 °C using a Turbiscan® MA 2000 (Formulaction, France). For these measurements, 7 mL of the double emulsion was added into a flat-bottomed cylindrical glass tube and scanned from the bottom to the top by a light source (pulsed near infra-red, 850 nm). The optical properties of the dispersion along the glass tube at different times provides a curve considered as a "macroscopic fingerprint". Data were obtained from backscattering (BS, %) of $W_1/O/W_2$ at 0, 3, 7, 15 and 21 days during storage at 4 °C.

2.7. Encapsulation efficiency and stability of MPPE $W_1/O/W_2$ emulsions

The encapsulation efficiency (EE, %) of MPPE $W_1/O/W_2$ emulsions was measured as the total PC that remained in the inner emulsions (W_1/O) after the second emulsification step. The remained PC content was quantified after centrifugation and filtration processes of double emulsions (Day 0), as described by Tepsongkroh, Harnsilawat, Maisuthisakul, and Chantrapornchai (2015), with minor changes. First, double emulsion samples (50 g) were centrifuged at 13,600 x *g* at 4

°C for 15 minutes. After centrifugation, the lower aqueous layer was carefully removed using glass Pasteur pipettes and filtered through a 0.22 µm nylon membrane filter. Then, the recovered PC were determined by Folin-Ciocalteu method, as described by Mazzucotelli et al. (2017), and expressed as mg of gallic acid equivalents (GAE) per gram of emulsion, using a standard calibration curve. The total PC recovered (TP) from emulsions was then calculated using the following Equation (2):

$$TP = [TP_e - TP_C] \quad (2)$$

where TP_e is the total GAE found in MPPE $W_1/O/W_2$ emulsions after centrifugation, whereas TP_C is the GAE value in control $W_1/O/W_2$ after centrifugation. Hence, the EE (%) of emulsions was calculated by using these data in the following Equation (3):

$$EE\ (\%) = \left[1 - \frac{TP}{TP_{MPPCE}}\right] x\ 100 \quad (3)$$

where TP_{MPPE} is the total amount of GAE in the MPPE dissolved using the aqueous phase solution. Likewise, the encapsulation stability (ES, %) of MPPE $W_1/O/W_2$ emulsions was evaluated during storage at 4°C, as performed by Matos, Gutiérrez, Coca, and Pazos (2014). The ES was considered as the amount of PC that remained in the inner emulsion (W_1/O) during storage after centrifugation and filtration processes, and it was calculated using Equation (4):

$$ES(\%) = \left[1 - \frac{TP}{TP_{MPPCE}}\right] x\ 100 \quad (3)$$

Thus, the recovered aqueous layer of MPPE $W_1/O/W_2$ emulsions after centrifugation were taken at different days of storage and kept at -18°C until the measure of ES.

2.8. Confocal laser scanning microscopy (CLSM)

The microstructure of $W_1/O/W_2$ emulsions was evaluated using a confocal laser scanning microscope (Olympus FV1000 Spectral Confocal Microscope Olympus, Melville, NY) as performed by Aditya et al. (2015). In order to stain the already formed W/O droplet structures, 1 mL of the emulsion was dyed by adding 12 µL of

Nile red solution. The Nile red dye solution was prepared by dissolving Nile red (1 mg/mL) using polyethylene glycol. The $W_1/O/W_2$ emulsions were allowed to stand for 15 minutes before observing it in the microscope, to dye the oil phase properly. Sample fluorescence was collected using an excitation wavelength of 488 nm and collecting wavelengths of 523-650 nm. Images were captured using a 100x objective.

2.9. Statistical Analysis

All the experiments in this study were performed in duplicate, using at least three measurements by each analysis. All statistics were performed on a completely randomized design, analyzing differences among the mean values by one-way ANOVA and Tukey-Kramer multiple comparison test ($p < 0.05$) using the statistical software NCSS 2007.

3. Results and discussion

3.1. Inner water-in-oil (W_1/O) emulsions characterization

For its use as an encapsulation system, a low particle size of inner W_1/O emulsions is essential for the later production of stable double emulsion systems. As double emulsions' instability phenomena may occur by diverse physicochemical mechanisms (gravitational separation, coalescence, flocculation or Ostwald ripening), lowering the inner emulsions' particle size may increase emulsions' stability during storage conditions (Öztürk, 2017). Iyer et al. (2015) reported that the O/W emulsions with a lower particle size (< 0.25 µm) shown less variability after four weeks of storage. Thus, stable $W_1/O/W_2$ emulsions may provide proper protection of encapsulated bioactive compounds for longer periods.

Our results have shown that the lowest particle size in control and MPPE W_1/O emulsions (0.36 ± 0.01 µm and 0.38 ± 0.00 µm, respectively) were obtained after 3 min of ultrasound treatment at 100 µm of amplitude (**Table 1**). Similar behavior was reported by Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, and Martín-Belloso (2014), as they observed that the lowest size (4.3 ± 0.2 nm) of O/W emulsion systems was achieved at the higher amplitude and treatment time (100 µm and 3 min). However,

differences between emulsions' particle size after ultrasound treatments may be related either to the type of emulsion, surfactant, oil, or possible surfactants' molecular interactions at emulsions interface.

Interestingly, our results showed that during ultrasound treatment, the time had an effect on emulsions' particle size and span only above 60 µm of amplitude. Similarly, Mahdi Jafari, He, and Bhandari (2006) reported that O/W emulsions' particle size and span was getting smaller when increasing treatment time at 100 µm of amplitude. Therefore, at this ultrasound treatment conditions (100 µm of amplitude and 3 min), acoustic cavitation has broken water droplets, allowing them to accumulate enough surfactant to achieve the smaller particle size and lower span value. However, no differences were observed between control and MPPE W_1/O emulsions, suggesting that there are no molecular interactions between MPPE and the inner hydrophobic surfactant (PGPR) at the emulsions interface, that may prevent its encapsulation within W_1/O emulsions.

3.2. Water-in-oil-in-water (W_1/O/W_2) double emulsions formulation

During secondary emulsification, the surfactant selection may determine the emulsions physical characteristics, as well as the encapsulation efficiency (EE) and stability (ES) during storage conditions. In that sense, the results shown in **Figure 1** suggests that double emulsions' formulation using Tween 20 as the second surfactant, had the lowest particle size (3.68 ± 0.16 µm), but also the higher EE (98.65 ± 1.14 %). The EE is an important parameter that determines the efficiency of W_1/O/W_2 systems to retain MPPE within the inner aqueous phase of emulsions. Similar results were reported by Aditya et al. (2015) for catechin loaded emulsions, using Tween 80, achieving a particle size of 3.70 ± 0.2 µm and EE of 94 ± 2 %. Similarly, Matos et al. (2014) suggest that using different hydrophilic surfactants, the recovery yield values for encapsulated trans-resveratrol were 97.84 ± 2.96 % and 95.14 ± 3.37 using Tween 20 and Tween 80, respectively.

The findings of this study are similar to those reported by Giroux, Robitaille, and Britten (2016) in W_1/O/W_2 systems using sodium caseinate as hydrophilic surfactant and different oil phase compositions. These authors observed that the

highest EE (96.6 %) was found in emulsions made with mineral oil, which also had a higher particle size (6.25 µm) than those made with butter or linseed oil (3.97 and 3.52 µm, respectively). On the other hand, our results have shown that the lowest EE was found in emulsions using Tween 80, with 89.31 ± 1.07 %. A slightly lower EE (84 %) were reported by Vladisavljević, Shimizu, and Nakashima (2006) in emulsions produced by membrane emulsification, using Tween 80 as the second surfactant. Gharehbeglou et al. (2019) reported that the amount of Tween 80 used in a double nano-emulsions system, loaded with gallic acid, influences the encapsulation efficiency of the emulsions between 77.2 and 87.5 %.

3.3. Encapsulation stability and physicochemical changes of $W_1/O/W_2$ emulsions during storage

The effect of the outer surfactant on the double emulsion stability was monitored in terms of particle size, viscosity, turbidity, and ES for 21 days. Our results suggest that the different hydrophilic surfactants affected the emulsions´ particle size changes during storage conditions (**Figure 1**), being those using Tween (either 20 or 80) the systems of higher stability. However, even though particle size tended to decrease in both systems, only emulsions using Tween 80 showed significant changes after 21 days of storage at 4°C. Thus, the differences observed between the particle size of control $W_1/O/W_2$ emulsions using Tween 80 may be explained by simple water diffusion between W_1 and W_2, as the particle size varies along the emulsions days of storage.

Tamnak et al. (2016) reported similar particle size reduction in emulsions stabilized using a pectin-pea protein isolated conjugate and Tween 80 as the surfactant. These authors attributed this behavior to simultaneous transportation of dissolved molecules and water from W_1 to W_2 through the oil layer. Even when electrolytes, sugars or other osmotic pressure regulators are included in emulsions formulation, W_1 droplet expulsion might not be controlled, as some emulsifiers may enable or enhance transport phenomena (Garti, 1997). Also, possible interactions between antioxidants within MPPE and hydrophilic surfactants should also be considered to explain the differences between MPPE and control $W_1/O/W_2$ emulsions.

According to Losada-Barreiro, Sánchez-Paz, and Bravo-Díaz (2013), the HLB of non-ionic surfactants (Tween 20, 40, 80 and Span 20) and its volume fraction in O/W emulsions may promote the incorporation of hydrophilic antioxidants to interfacial regions. In comparison to our results, Gomes, Costa, de Assis Perrechil, and da Cunha (2016) reported that the presence of gallic acid modified the physical properties of O/W emulsions using Tween 20 as the surfactant, as they observed a lower mean droplet diameter and increased stability in O/W emulsions. Furthermore, Losada-Barreiro et al. (2013) suggest that when using high HLB surfactants (such as Tweens) at a surfactant volume fraction of 0.04, more than 90% of gallic acid is located at the interfacial region of O/W emulsions. This positioning of antioxidants may be relevant to enhance the stability of emulsions, as it is suggested that antioxidants in food products should be located where the oxidation processes start, that is in emulsions, at the oil-water interface (González, Medina, Maldonado, Lucas, & Morales, 2015).

Therefore, the use of lower HLB surfactants may ease the incorporation of hydrophobic antioxidant molecules to the interfacial region, while preventing the incorporation of hydrophilic antioxidants (such as gallic acid). In our study, lecithin is the surfactant of lower HLB value (8), where its use in double emulsions suggest higher free energy and lower amounts of hydrophilic antioxidants at the O/W interfacial region, which results in less stable emulsions. The MPPE antioxidants, specifically gallic acid, may be positioned at the oil-water interface of $W_1/O/W_2$ emulsions using Tween (either 20 or 80) as hydrophilic surfactant. In addition, the presence of gallic acid at the interfacial region of emulsions has been reported to contribute positively to their stability over storage time (Di Mattia, C. D., Sacchetti, Mastrocola, & Pittia, 2009). Thus, this may explain the differences observed in our study, as those MPPE-loaded $W_1/O/W_2$ emulsions had higher stability than control emulsions.

Emulsions using lecithin as surfactant had an opposite behavior compared to that observed in emulsions using either Tween 20 or Tween 80. In this case, particle size increased 4.5-fold (from 6.75 to 30 µm) its initial value after day 1 and a 12-

fold increase (from 6.75 to 82 µm) after 21 days of storage. Scherze, Knoth, and Muschiolik (2006) reported that electrolytes present in emulsions might have an important effect on lecithin emulsification properties, as its main function is to provide an electrostatic repulsion barrier to the emulsion droplets. In addition, Luo et al. (2017) described that when multiple emulsions are under high NaCl concentration, particle size increase, leading to oil droplet coalescence. Thus, $W_1/O/W_2$ emulsions using lecithin may require lower NaCl concentrations. However, when no electrolytes are added to $W_1/O/W_2$ emulsions, it becomes difficult to control Laplace and osmotic pressures, leading to excessive and undesired water transport between W_1 and W_2.

Furthermore, viscosity can also be considered as a stability parameter for $W_1/O/W_2$ as, according to Stoke's law, the emulsions viscosity decrease when W_1 is released, or creaming have occurred (Lamba, Sathish, & Sabikhi, 2015). Emulsions using Tween 80 and lecithin showed more stable viscosity values during storage, while MPPE $W_1/O/W_2$ emulsions using Tween 20 had the highest apparent viscosity values, and also the more noticeable decrease during storage conditions (see **Figure 2**). Even though differences between MPPE $W_1/O/W_2$ and control emulsions viscosity (11.06 and 3.54 mPa•S, respectively) were not fully elucidated in this study, we hypothesized that some phenolic species within MPPE might interact with Tween 20 absorbed at the O/W interface. In agreement to our hypothesis, Di Mattia, C. D. et al. (2009) reported that catechin molecules, also present in MPPE, had an interfacial localization in emulsions using Tween 20, increasing interfacial activity while showing possible surfactant-antioxidant interactions that lead to the instability of emulsions. Thus, as a result of increased energy at the interfacial region of emulsions, flocculation of droplets may have occurred, becoming more susceptible to gravitational forces as days of storage passed.

On the other hand, as instability phenomena lead to changes in optical properties, $W_1/O/W_2$ stability measured by BS profile along storage was performed. **Figure 3** shows BS profiles of $W_1/O/W_2$ using either Tween 20, Tween 80 or lecithin to

identify instability phenomena matching the previously described properties (particle size and viscosity) with its “macroscopic fingerprint” at a given time. Changes in BS profiles during storage can be interpreted as follows: a) BS% decrease in the bottom of the tube represents clarification; b) BS% changes in middle zone are related to either flocculation or coalescence phenomena; c) BS% decrease in bottom of the tube and BS% increase in top of the tube corresponds to creaming (Fioramonti, Arzeni, Pilosof, Rubiolo, & Santiago, 2015). Thus, our results suggest that $W_1/O/W_2$ using Tween 80 and lecithin were the most vulnerable to creaming, which is more noticeable on MPPE $W_1/O/W_2$ emulsions, during storage conditions. Likewise, clarification and flocculation/coalescence was more evident in MPPE than control $W_1/O/W_2$ emulsions when using Tween 20, which supports the previously mentioned possible interactions between MPPE and Tween 20 molecules.

Stability results of emulsions using Tween 20 (particle size, viscosity, BS%) suggests that during storage, either control or MPPE $W_1/O/W_2$ emulsions may be flocculating, as their particle size decreased, while agglomeration into larger particles may take place during storage conditions due to clarification and viscosity decrease. On the other hand, in emulsions using lecithin the coalescence of particles occurred due to phase separation leading to creaming appearance. Creaming is one of the most common instability phenomena, and it occurs by an upward movement of big droplets as a result of density differences with the continuous phase. Furthermore, Tween 80 optical behavior does not match coalescence criteria, as particle size decreased in either MPPE or control $W_1/O/W_2$ emulsions, which was proven by a laser light scattering technique during storage. As a result of these particle size decrease, higher values of span were observed during storage conditions. This behavior may be attributed to excessive flocculation of droplets, which could also result in phase separation leading to creaming phenomena (Owens, Griffin, Khouryieh, & Williams, 2018).

Furthermore, Krstonošić, Dokić, Dokić, and Dapčević (2009) observed that the use of Tween 80 as the stabilizer in O/W emulsions resulted in unstable emulsions,

susceptible to creaming and coalescence. In addition, Jiao and Burgess (2003) showed that using high concentrations of Tween 80 may decrease emulsions' stability. These results suggest that high concentrations of hydrophilic surfactants, Tween 80 in our case, may cause droplets disruption. Hence, an explanation for the instability signs of emulsions using Tween 80 may be due to the critical micellar concentration of Tween 80, which is exceeded above 1% of concentration. Thus, the emulsion' interface is populated by surfactant molecules, and the excess spontaneously self-assembles into micelles in the continuous phase to further reduce the interfacial tension (Raikos, Duthie, & Ranawana, 2017). However, in this study, a fixed concentration of 2% w/w was used for all $W_1/O/W_2$ emulsions.

Finally, ES, which followed the initial EE (day 0) of the emulsions, was used as an indicator of emulsions stability and effectiveness as encapsulation systems for MPPE, confirming the previously discussed instability phenomena occurred. Although the emulsions' initial EE shown values (between 89-98%) similar to those reported by Tepsongkroh et al. (2015) for encapsulated mango seed kernel extracts in a $W_1/O/W_2$ emulsion, trends of ES versus time followed a similar trend to that reported by Matos et al. (2014), by decreasing during storage conditions. Our results have shown that, even though the emulsions using Tween 20 and lecithin had the highest EE values, their ES rapidly decay along storage.

Decay in the ES values, along with a particle size decrease, might be attributed to the diffusion of the MPPE from the W_1 to the W_2 phase. The diffusion between phases become clearer in emulsions using lecithin as the surfactant, as continuous decay in ES and a particle size increase were observed since day 1. According to Wen and Papadopoulos (2001), the diffusion between phases may be explained by the breakdown of the oil globules due to the loss of the surfactants properties. Moreover, the presence of catechin within MPPE may have caused a competitive displacement of surfactants in emulsions using Tween 20 and lecithin, as suggested by Di Mattia, Carla D., Sacchetti, Mastrocola, Sarker, and Pittia (2010). Thus, an increase of interfacial tension at the oil/water interface may be occurring

in emulsions using lecithin and tween 20, reducing the surfactants adsorption density.

Conversely, the emulsions prepared using Tween 80 showed the lowest EE but kept better ES values during storage. Aditya et al. (2015) observed minor losses and a higher ES of catechin in $W_1/O/W_2$ emulsions using Tween 80 as the surfactant, from day 0 (94%) to day 15 (91%) of storage at 4°C. These results suggest that minor leakage between phases occur in emulsions using Tween 80. However, as either EE and ES were measured in terms of total phenolics by Folin-Ciocalteu method, less oxidation/reduction reactions may be occurring at the O/W interface region when using Tween 80 as the surfactant. In that case, the antioxidants within MPPE may be acting to prevent or stop the lipid oxidation at the interfacial region of emulsions, losing their ability to react by the Folin-Ciocalteu method. The protective role of MPPE towards lipids auto-oxidation in emulsions may be related to either antioxidants polarity and the surfactant-antioxidant interactions, as suggested by Di Mattia, C. D. et al. (2009), or even to the PC antioxidant activity mechanism.

3.4. Microstructure of $W_1/O/W_2$ emulsions

The microstructure of MPPE and control $W_1/O/W_2$ emulsions was visualized by CLSM, as shown in **Figure 4**. Images captured at day 0 confirms the small particle size of emulsions with droplets below 10 µm, and droplets flocculation in emulsions using Tween 80. As previously discussed, this excessive flocculation in Tween 80 emulsions may be explained by the high surfactant concentration, which was above its critical micelle concentration.

As CLSM images show, flocculation in MPPE $W_1/O/W_2$ emulsions using Tween 80 occurs since day 0 and reaches excessive levels that lead to creaming after 21 days of storage. Above critical flocculation concentrations (CFC), creaming may occur as a thick oil layer formed in emulsions due to an upward movement of droplets (Bai et al., 2017). This behavior is considerably different between MPPE and control $W_1/O/W_2$ emulsions using Tween 80, as creaming in control $W_1/O/W_2$ may be due to droplet coalescence, instead of reaching a CFC. These results

suggest that differences may be related to changes in the interfacial tension of emulsions caused by MPPE. As Terjung, Löffler, Gibis, Hinrichs, and Weiss (2012) reported, PC may not be successfully incorporated into stable emulsions if loading is excessively high. According to these assertions, it seems that some PC within MPPE move through the W_1 to the O/W_2 interface, promoting particle flocculation due to interfacial interactions with Tween 80 molecules.

On the other hand, CLSM images of $W_1/O/W_2$ using lecithin, also corroborates the previously discussed results. Even when the initial conditions of $W_1/O/W_2$ seemed to have a comparable particle size and span value to those in emulsions using Tween 20 or 80, lecithin had poor stability as a surfactant during storage, with notorious phase separation after 21 days. These CLSM images shown bigger oil droplets of non-spherical shape, which is an indicator of a lack surfactant adsorbed at O/W droplets interface. Similar to our study, Züge, Haminiuk, Maciel, Silveira, and Scheer (2013), reported that emulsions prepared with lecithin are less stable than those prepared using Tween 80. Even though MPPE $W_1/O/W_2$ emulsions had better physical appearance and particle size than control emulsions using lecithin as a the surfactant at day 0, CLSM images shown that both $W_1/O/W_2$ emulsions had a similar droplet increase as a product of droplets coalescence after 21 days of storage. Therefore, emulsions that use lecithin as hydrophilic surfactant need to include either co-surfactant molecules or stabilizing agents to reduce particle collision and instability phenomena during storage conditions.

4. Conclusion

This study demonstrates the feasibility of MPPE encapsulation using emulsion-based systems, as it was successfully incorporated into long-term stable $W_1/O/W_2$ emulsions. Even though similar trends were observed in physical stability (size, viscosity, optical appearance), our study showed that the highest ES was observed in emulsions with Tween 80 as the surfactant. Despite the higher EE of emulsions with Tween 20 and lecithin, they had lower ES along storage. Also, the differences between control and MPPE-loaded emulsions suggests the movement of PC from W_1 through the oil phase and their positioning at the O/W interface of emulsions.

Thus, PC-surfactants interactions may influence the physical stability of emulsions. Further studies about emulsions' stability and release of loaded PC during gastrointestinal conditions may provide valuable insights towards the formulation of new functional food products containing bioactive ingredients from agroindustrial by-products.

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6. References

Aditya, N., Aditya, S., Yang, H.-J., Kim, H. W., Park, S. O., Lee, J., & Ko, S. (2015). Curcumin and catechin co-loaded water-in-oil-in-water emulsion and its beverage application. *Journal of Functional Foods, 15*, 35-43. doi: https://doi.org/10.1016/j.jff.2015.03.013

Ajila, C. M., Aalami, M., Leelavathi, K., & Rao, U. J. S. P. (2010). Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. *Innovative Food Science & Emerging Technologies, 11*(1), 219-224. doi: https://doi.org/10.1016/j.ifset.2009.10.004

Andrade, J., & Corredig, M. (2016). Vitamin D3 and phytosterols affect the properties of polyglycerol polyricinoleate (PGPR) and protein interfaces. *Food Hydrocolloids, 54*, 278-283. doi: https://doi.org/10.1016/j.foodhyd.2015.10.001

Artiga-Artigas, M., Acevedo-Fani, A., & Martín-Belloso, O. (2017). Effect of sodium alginate incorporation procedure on the physicochemical properties of nanoemulsions. *Food Hydrocolloids, 70*, 191-200. doi: http://dx.doi.org/10.1016/j.foodhyd.2017.04.006

Bai, L., Liu, F., Xu, X., Huan, S., Gu, J., & McClements, D. J. (2017). Impact of polysaccharide molecular characteristics on viscosity enhancement and depletion flocculation. *Journal of Food Engineering, 207*, 35-45. doi: http://dx.doi.org/10.1016/j.jfoodeng.2017.03.021

Blancas-Benitez, F. J., de Jesús Avena-Bustillos, R., Montalvo-González, E., Sáyago-Ayerdi, S. G., & H. McHugh, T. (2015). Addition of dried 'Ataulfo' mango (Mangifera indica L) by-products as a source of dietary fiber and polyphenols in starch molded mango snacks. [journal

article]. *Journal of Food Science and Technology, 52*(11), 7393-7400. doi: 10.1007/s13197-015-1855-7

de Almeida Paula, D., Mota Ramos, A., Basílio de Oliveira, E., Maurício Furtado Martins, E., Augusto Ribeiro de Barros, F., Cristina Teixeira Ribeiro Vidigal, M., de Almeida Costa, N., & Tatagiba da Rocha, C. (2018). Increased thermal stability of anthocyanins at pH 4.0 by guar gum in aqueous dispersions and in double emulsions W/O/W. *International Journal of Biological Macromolecules, 117*, 665-672. doi: https://doi.org/10.1016/j.ijbiomac.2018.05.219

Di Mattia, C. D., Sacchetti, G., Mastrocola, D., & Pittia, P. (2009). Effect of phenolic antioxidants on the dispersion state and chemical stability of olive oil O/W emulsions. *Food Research International, 42*(8), 1163-1170. doi: https://doi.org/10.1016/j.foodres.2009.05.017

Di Mattia, C. D., Sacchetti, G., Mastrocola, D., Sarker, D. K., & Pittia, P. (2010). Surface properties of phenolic compounds and their influence on the dispersion degree and oxidative stability of olive oil O/W emulsions. *Food Hydrocolloids, 24*(6), 652-658. doi: https://doi.org/10.1016/j.foodhyd.2010.03.007

Faridi Esfanjani, A., Jafari, S. M., & Assadpour, E. (2017). Preparation of a multiple emulsion based on pectin-whey protein complex for encapsulation of saffron extract nanodroplets. *Food Chemistry, 221*, 1962-1969. doi: https://doi.org/10.1016/j.foodchem.2016.11.149

Fioramonti, S. A., Arzeni, C., Pilosof, A. M., Rubiolo, A. C., & Santiago, L. G. (2015). Influence of freezing temperature and maltodextrin concentration on stability of linseed oil-in-water multilayer emulsions. *Journal of Food Engineering, 156*, 31-38. doi: https://doi.org/10.1016/j.jfoodeng.2015.01.013

Garti, N. (1997). Double emulsions — scope, limitations and new achievements. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 123*, 233-246. doi: http://dx.doi.org/10.1016/S0927-7757(96)03809-5

Gharehbeglou, P., Jafari, S. M., Hamishekar, H., Homayouni, A., & Mirzaei, H. (2019). Pectin-whey protein complexes vs. small molecule surfactants for stabilization of double nano-emulsions as novel bioactive delivery systems. *Journal of Food Engineering, 245*, 139-148. doi: https://doi.org/10.1016/j.jfoodeng.2018.10.016

Giroux, H. J., Robitaille, G., & Britten, M. (2016). Controlled release of casein-derived peptides in the gastrointestinal environment by encapsulation in water-in-oil-in-water double emulsions. *LWT-Food Science and Technology, 69*, 225-232. doi: https://doi.org/10.1016/j.lwt.2016.01.050

Gomes, A., Costa, A. L. R., de Assis Perrechil, F., & da Cunha, R. L. (2016). Role of the phases composition on the incorporation of gallic acid in O/W and W/O emulsions. *Journal of Food Engineering, 168*, 205-214. doi: https://doi.org/10.1016/j.jfoodeng.2015.07.041

González, M. J., Medina, I., Maldonado, O. S., Lucas, R., & Morales, J. C. (2015). Antioxidant activity of alkyl gallates and glycosyl alkyl gallates in fish oil in water emulsions: Relevance of their surface active properties and of the type of emulsifier. *Food Chemistry, 183*, 190-196. doi: https://doi.org/10.1016/j.foodchem.2015.03.035

Guerra-Rosas, M. I., Morales-Castro, J., Ochoa-Martínez, L. A., Salvia-Trujillo, L., & Martín-Belloso, O. (2016). Long-term stability of food-grade nanoemulsions from high methoxyl pectin containing essential oils. *Food Hydrocolloids, 52*, 438-446. doi: https://doi.org/10.1016/j.foodhyd.2015.07.017

Iyer, V., Cayatte, C., Guzman, B., Schneider-Ohrum, K., Matuszak, R., Snell, A., Rajani, G. M., McCarthy, M. P., & Muralidhara, B. (2015). Impact of formulation and particle size on stability and immunogenicity of oil-in-water emulsion adjuvants. *Human Vaccines &*

Immunotherapeutics, 11(7), 1853-1864. doi: http://dx.doi.org/10.1080/21645515.2015.1046660

Jiang, J., Jin, Y., Liang, X., Piatko, M., Campbell, S., Lo, S. K., & Liu, Y. (2018). Synergetic interfacial adsorption of protein and low-molecular-weight emulsifiers in aerated emulsions. *Food Hydrocolloids, 81*, 15-22. doi: https://doi.org/10.1016/j.foodhyd.2018.02.038

Jiao, J., & Burgess, D. J. (2003). Rheology and stability of water-in-oil-in-water multiple emulsions containing Span 83 and Tween 80. [journal article]. *American Association of Pharmaceutical Scientists, 5*(1), 62-73. doi: https://doi.org/10.1208/ps050107

Katsouli, M., Polychniatou, V., & Tzia, C. (2017). Influence of surface-active phenolic acids and aqueous phase ratio on w/o nano-emulsions properties; model fitting and prediction of nano-emulsions oxidation stability. *Journal of Food Engineering, 214*, 40-46. doi: https://doi.org/10.1016/j.jfoodeng.2017.06.017

Kaur, J., Whitson, A., Ashton, J., Katopo, L., & Kasapis, S. (2018). Effect of ultra high temperature processing and storage conditions on phenolic acid, avenanthramide, free fatty acid and volatile profiles from Australian oat grains. *Bioactive Carbohydrates and Dietary Fibre, 15*, 21-29. doi: https://doi.org/10.1016/j.bcdf.2016.09.002

Krook, M. A., & Hagerman, A. E. (2012). Stability of polyphenols epigallocatechin gallate and pentagalloyl glucose in a simulated digestive system. *Food Research International, 49*(1), 112-116. doi: https://doi.org/10.1016/j.foodres.2012.08.004

Krstonošić, V., Dokić, L., Dokić, P., & Dapčević, T. (2009). Effects of xanthan gum on physicochemical properties and stability of corn oil-in-water emulsions stabilized by polyoxyethylene (20) sorbitan monooleate. *Food Hydrocolloids, 23*(8), 2212-2218. doi: http://dx.doi.org/10.1016/j.foodhyd.2009.05.003

Lamba, H., Sathish, K., & Sabikhi, L. (2015). Double Emulsions: Emerging Delivery System for Plant Bioactives. [Review]. *Food and Bioprocess Technology, 8*(4), 709-728. doi: https://doi.org/10.1007/s11947-014-1468-6

Losada-Barreiro, S., Sánchez-Paz, V., & Bravo-Díaz, C. (2013). Effects of emulsifier hydrophile–lipophile balance and emulsifier concentration on the distributions of gallic acid, propyl gallate, and α-tocopherol in corn oil emulsions. *Journal of Colloid and Interface Science, 389*(1), 1-9. doi: https://doi.org/10.1016/j.jcis.2012.07.036

Luo, S., Zhang, C., Hu, L., Zhang, Z., Niu, Y., & Zhang, W. (2017). Stability and rheology of three types of W/O/W multiple emulsions emulsified with lecithin. *Journal of Dispersion Science and Technology, 38*(11), 1530-1535. doi: https://doi.org/10.1080/01932691.2016.1259074

Macedo, J. P., Fernandes, L. L., Formiga, F. R., Reis, M. F., Júnior, T. N., Soares, L. A., & Egito, E. S. T. (2006). Micro-emultocrit technique: a valuable tool for determination of critical HLB value of emulsions. *American Association of Pharmaceutical Scientists, 7*(1), E146-E152. doi: https://dx.doi.org/10.1208%2Fpt070121

Mahdi Jafari, S., He, Y., & Bhandari, B. (2006). Nano-Emulsion Production by Sonication and Microfluidization—A Comparison. *International Journal of Food Properties, 9*(3), 475-485. doi: https://doi.org/10.1080/10942910600596464

Matos, M., Gutiérrez, G., Coca, J., & Pazos, C. (2014). Preparation of water-in-oil-in-water (W1/O/W2) double emulsions containing trans-resveratrol. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 442*, 69-79. doi: http://dx.doi.org/10.1016/j.colsurfa.2013.05.065

Mattila, P., & Kumpulainen, J. (2002). Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. *Journal of Agricultural and Food Chemistry, 50*(13), 3660-3667. doi: https://doi.org/10.1021/jf020028p

Mazzucotelli, C. A., González-Aguilar, G. A., Villegas-Ochoa, M. A., Domínguez-Avila, A. J., Ansorena, M. R., & Di Scala, K. C. (2017). Chemical characterization and functional properties of selected leafy vegetables for innovative mixed salads. *Journal of Food Biochemistry*. doi: https://doi.org/10.1111/jfbc.12461

Miyague, L., Macedo, R. E. F., Meca, G., Holley, R. A., & Luciano, F. B. (2015). Combination of phenolic acids and essential oils against Listeria monocytogenes. *LWT - Food Science and Technology, 64*(1), 333-336. doi: https://doi.org/10.1016/j.lwt.2015.05.055

Momeny, E., Mirhosseini, H., & Sarker, M. Z. I. (2017). Effect of Medium-High Energy Emulsification Condition on Physicochemical Properties of β-Sitosterol Multiple Emulsion. [journal article]. *Food and Bioprocess Technology, 10*(9), 1642-1654. doi: http://dx.doi.org/10.1007/s11947-017-1932-1

Okino Delgado, C. H., & Fleuri, L. F. (2016). Orange and mango by-products: Agro-industrial waste as source of bioactive compounds and botanical versus commercial description—A review. *Food Reviews International, 32*(1), 1-14. doi: 10.1080/87559129.2015.1041183

Owens, C., Griffin, K., Khouryieh, H., & Williams, K. (2018). Creaming and oxidative stability of fish oil-in-water emulsions stabilized by whey protein-xanthan-locust bean complexes: Impact of pH. *Food Chemistry, 239*, 314-322. doi: https://doi.org/10.1016/j.foodchem.2017.06.096

Öztürk, B. (2017). Nanoemulsions for food fortification with lipophilic vitamins: Production challenges, stability, and bioavailability. [Review]. *European Journal of Lipid Science and Technology, 119*(7), 18. doi: http://dx.doi.org/10.1002/ejlt.201500539

Pacheco-Ordaz, R., Antunes-Ricardo, M., Gutiérrez-Uribe, J., & González-Aguilar, G. (2018). Intestinal Permeability and Cellular Antioxidant Activity of Phenolic Compounds from Mango (Mangifera indica cv. Ataulfo) Peels. *International Journal of Molecular Sciences, 19*(2), 514. doi: http://dx.doi.org/10.3390/ijms19020514

Palafox-Carlos, H., Yahia, E., Islas-Osuna, M., Gutierrez-Martinez, P., Robles-Sánchez, M., & González-Aguilar, G. (2012). Effect of ripeness stage of mango fruit (Mangifera indica L., cv. Ataulfo) on physiological parameters and antioxidant activity. *Scientia Horticulturae, 135*, 7-13.

Raikos, V., Duthie, G., & Ranawana, V. (2017). Comparing the efficiency of different food-grade emulsifiers to form and stabilise orange oil-in-water beverage emulsions: influence of emulsifier concentration and storage time. *International Journal of Food Science & Technology, 52*(2), 348-358. doi: http://dx.doi.org/10.1111/ijfs.13286

Ramírez-Maganda, J., Blancas-Benítez, F. J., Zamora-Gasga, V. M., García-Magaña, M. d. L., Bello-Pérez, L. A., Tovar, J., & Sáyago-Ayerdi, S. G. (2015). Nutritional properties and phenolic content of a bakery product substituted with a mango (Mangifera indica) 'Ataulfo' processing by-product. *Food Research International, 73*, 117-123. doi: https://doi.org/10.1016/j.foodres.2015.03.004

Ribas-Agustí, A., Martín-Belloso, O., Soliva-Fortuny, R., & Elez-Martínez, P. (2018). Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods. *Critical Reviews in Food Science and Nutrition, 58*(15), 2531-2548. doi: 10.1080/10408398.2017.1331200

Rothwell, J. A., Medina-Remón, A., Pérez-Jiménez, J., Neveu, V., Knaze, V., Slimani, N., & Scalbert, A. (2015). Effects of food processing on polyphenol contents: A systematic analysis using Phenol-Explorer data. *Molecular Nutrition & Food Research, 59*(1), 160-170. doi: 10.1002/mnfr.201400494

Salvia-Trujillo, L., Rojas-Graü, M. A., Soliva-Fortuny, R., & Martín-Belloso, O. (2014). Impact of microfluidization or ultrasound processing on the antimicrobial activity against Escherichia

coli of lemongrass oil-loaded nanoemulsions. *Food Control, 37*, 292-297. doi: http://dx.doi.org/10.1016/j.foodcont.2013.09.015

Sáyago-Ayerdi, S. G., Moreno-Hernández, C. L., Montalvo-González, E., García-Magaña, M. L., de Oca, M. M.-M., Torres, J. L., & Pérez-Jiménez, J. (2013). Mexican 'Ataulfo'mango (Mangifera indica L) as a source of hydrolyzable tannins. Analysis by MALDI-TOF/TOF MS. *Food Research International, 51*(1), 188-194. doi: https://doi.org/10.1016/j.foodres.2012.11.034

Scherze, I., Knoth, A., & Muschiolik, G. (2006). Effect of Emulsification Method on the Properties of Lecithin-and PGPR-Stabilized Water-in-Oil-Emulsions. *Journal of Dispersion Science and Technology, 27*(4), 427-434. doi: http://dx.doi.org/10.1080/01932690500357081

Schmidts, T., Dobler, D., Nissing, C., & Runkel, F. (2009). Influence of hydrophilic surfactants on the properties of multiple W/O/W emulsions. *Journal of Colloid and Interface Science, 338*(1), 184-192. doi: https://doi.org/10.1016/j.jcis.2009.06.033

Tamnak, S., Mirhosseini, H., Tan, C. P., Amid, B. T., Kazemi, M., & Hedayatnia, S. (2016). Encapsulation properties, release behavior and physicochemical characteristics of water-in-oil-in-water (W/O/W) emulsion stabilized with pectin–pea protein isolate conjugate and Tween 80. *Food Hydrocolloids, 61*, 599-608. doi: http://dx.doi.org/10.1016/j.foodhyd.2016.06.023

Tepsongkroh, B., Harnsilawat, T., Maisuthisakul, P., & Chantrapornchai, W. (2015). Influence of Polyglycerol Polyricinoleate and Biopolymers on Physical Properties and Encapsulation Efficiency of Water-in-Oil-in-Water Emulsions Containing Mango Seed Kernel Extract. *Journal of Dispersion Science and Technology, 36*(8), 1126-1133. doi: http://dx.doi.org/10.1080/01932691.2014.956116

Terjung, N., Löffler, M., Gibis, M., Hinrichs, J., & Weiss, J. (2012). Influence of droplet size on the efficacy of oil-in-water emulsions loaded with phenolic antimicrobials. *Food & Function, 3*(3), 290-301. doi: http://dx.doi.org/10.1039/C2FO10198J

Velderrain-Rodríguez, G., Ovando-Martínez, M., Villegas-Ochoa, M., Ayala-Zavala, J. F., Wall-Medrano, A., Álvarez-Parrilla, E., Madera-Santana, T. J., Astiazarán-García, H., Tortoledo-Ortiz, O., & González-Aguilar, G. A. (2015). Antioxidant Capacity and Bioaccessibility of Synergic Mango (cv. Ataulfo) Peel Phenolic Compounds in Edible Coatings Applied to Fresh-Cut Papaya. *Food and Nutrition Sciences, 6*(3), 365. doi: https://doi.org/10.4236/fns.2015.63037

Velderrain-Rodriguez, G., Quiros-Sauceda, A., Mercado-Mercado, G., Ayala-Zavala, J. F., Astiazaran-Garcia, H., Robles-Sanchez, R. M., Wall-Medrano, A., Sayago-Ayerdi, S., & Gonzalez-Aguilar, G. A. (2016). Effect of dietary fiber on the bioaccessibility of phenolic compounds of mango, papaya and pineapple fruits by an in vitro digestion model. [Article]. *Food Science and Technology, 36*(2), 188-194. doi: http://dx.doi.org/10.1590/1678-457x.6729

Velderrain-Rodríguez, G. R., Torres-Moreno, H., Villegas-Ochoa, M., Ayala-Zavala, J. F., Robles-Zepeda, R., Wall-Medrano, A., & González-Aguilar, G. A. (2018). Gallic Acid Content and an Antioxidant Mechanism Are Responsible for the Antiproliferative Activity of 'Ataulfo' Mango Peel on LS180 Cells. *Molecules, 23*(3), 695. doi: https://doi.org/10.3390/molecules23030695

Vladisavljević, G. T., Shimizu, M., & Nakashima, T. (2006). Production of multiple emulsions for drug delivery systems by repeated SPG membrane homogenization: Influence of mean pore size, interfacial tension and continuous phase viscosity. *Journal of Membrane Science, 284*(1), 373-383. doi: https://doi.org/10.1016/j.memsci.2006.08.003

Wen, L., & Papadopoulos, K. D. (2001). Effects of Osmotic Pressure on Water Transport in W1/O/W2 Emulsions. *Journal of Colloid and Interface Science, 235*(2), 398-404. doi: https://doi.org/10.1006/jcis.2000.7384

Williamson, G., & Holst, B. (2008). Dietary reference intake (DRI) value for dietary polyphenols: are we heading in the right direction? *British Journal of Nutrition, 99*(S3), S55-S58. doi: 10.1017/S0007114508006867

Zhang, W., Zhai, X., Ou, W., Song, L., & Zhang, Q. (2018). Influencing factors of multiple emulsions formed by one-step emulsification. [journal article]. *Colloid and Polymer Science, 296*(2), 259-269. doi: 10.1007/s00396-017-4232-4

Züge, L. C. B., Haminiuk, C. W. I., Maciel, G. M., Silveira, J. L. M., & Scheer, A. d. P. (2013). Catastrophic inversion and rheological behavior in soy lecithin and Tween 80 based food emulsions. *Journal of Food Engineering, 116*(1), 72-77. doi: https://doi.org/10.1016/j.jfoodeng.2012.12.008

FIGURES

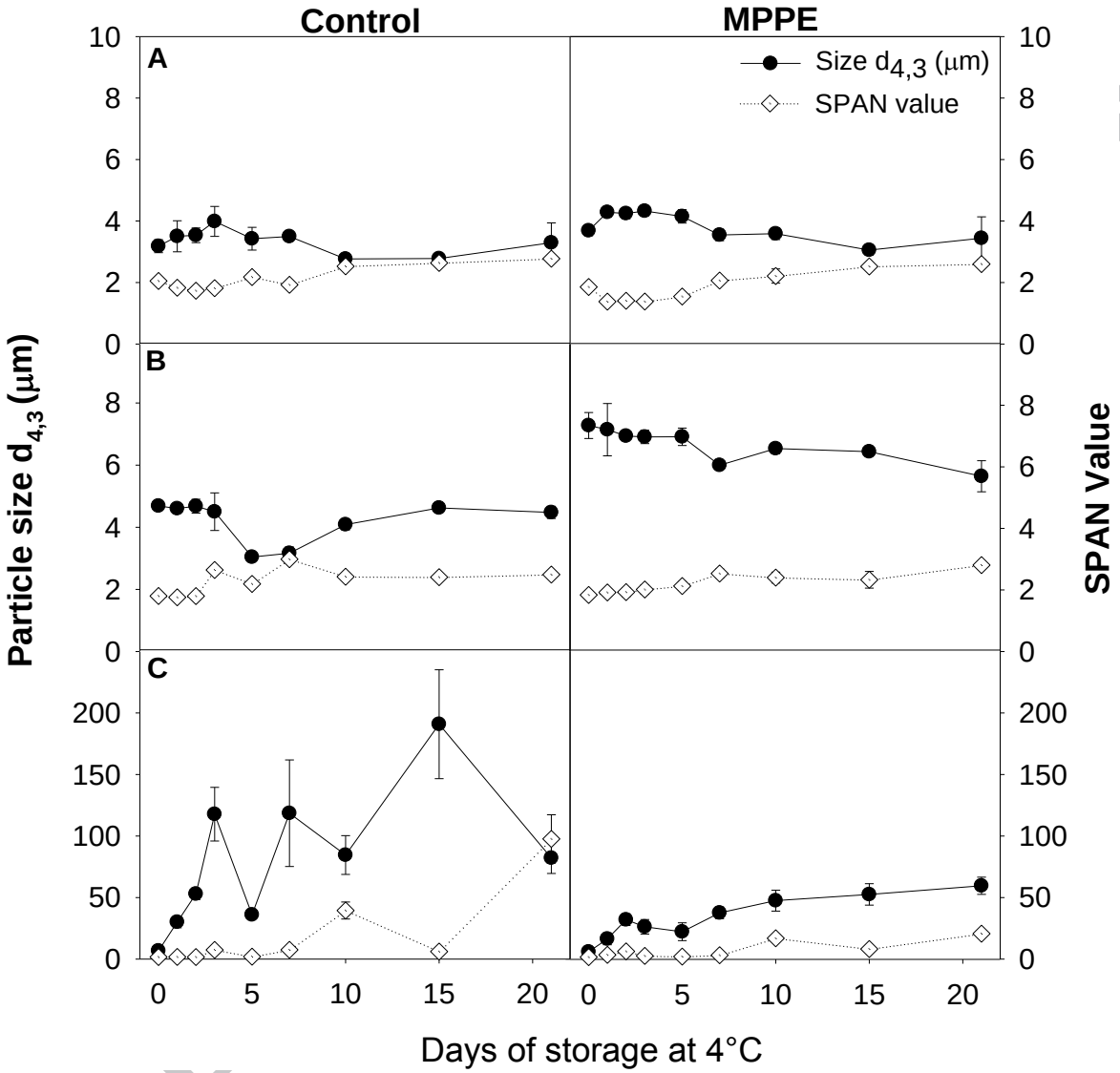

Figure 1. Size changes of $W_1/O/W_2$ emulsions during storage conditions. MPPE = Mango peel phenolic-rich extract. A) Tween 20; B) Tween 80; C) Lecithin; Data is expressed as volume-weighted average ($d_{4,3}$) and size distribution in terms of span values. All data reported are mean ± SD (n=3).

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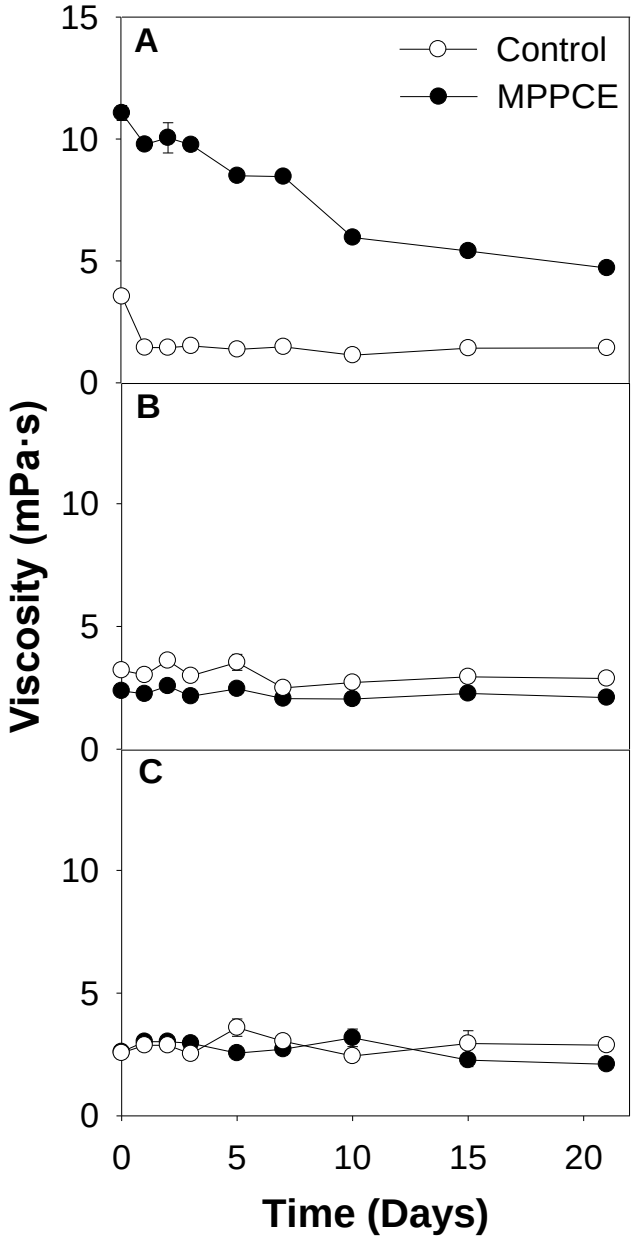

Figure 2. Apparent viscosity of $W_1/O/W_2$ emulsions during storage at 4 °C. MPPE = Mango peel phenolic-rich extract. A) Tween 20; B) Tween 80; C) Lecithin. All data reported are mean ± SD (n=3).

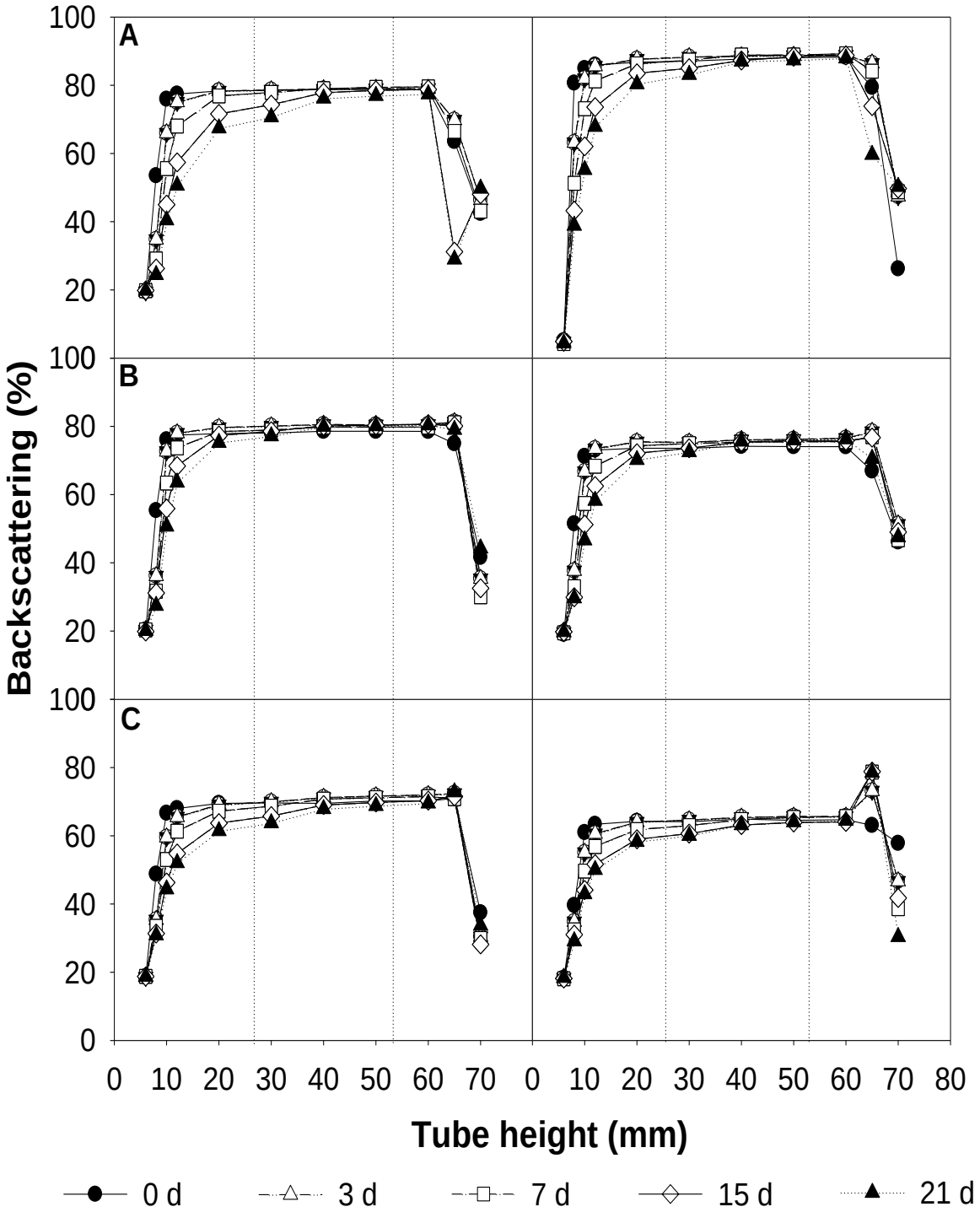

Figure 3. Backscattering profiles of $W_1/O/W_2$ emulsions during 21 days of storage at 4 °C. MPPE = Mango peel phenolic-rich extract. A) Tween 20; B) Tween 80; C) Lecithin. The graphics represent changes in BS% considering 3 main zones of the

glass tube: Bottom (left side), middle, and top of the tube (right side). Measures were performed at 0, 3, 7, 15 and 21 days of storage at 4 °C.

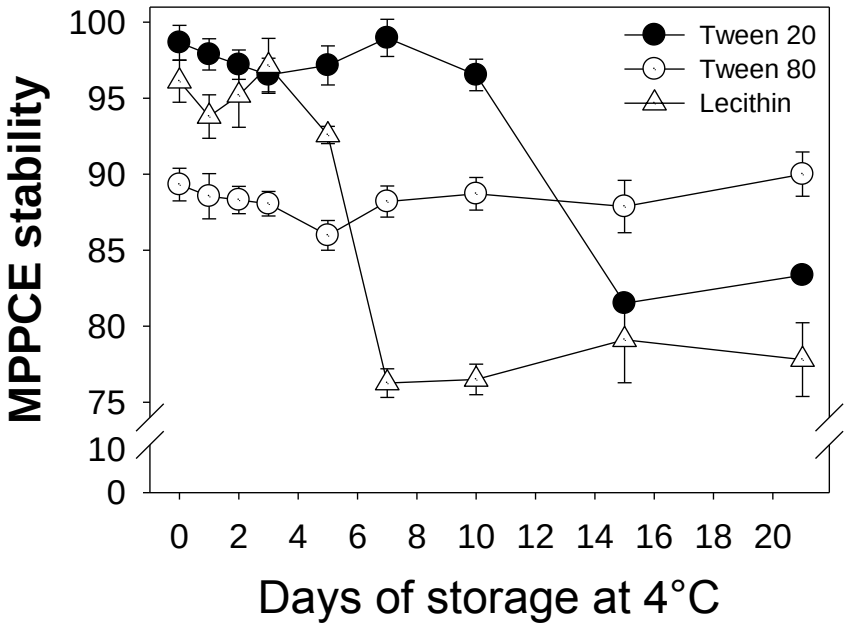

Figure 4. Encapsulation stability of mango peel phenolic-rich extract (MPPE) $W_1/O/W_2$ emulsions using Tween 20, Tween 80 and lecithin during 21 days of storage at 4 °C.

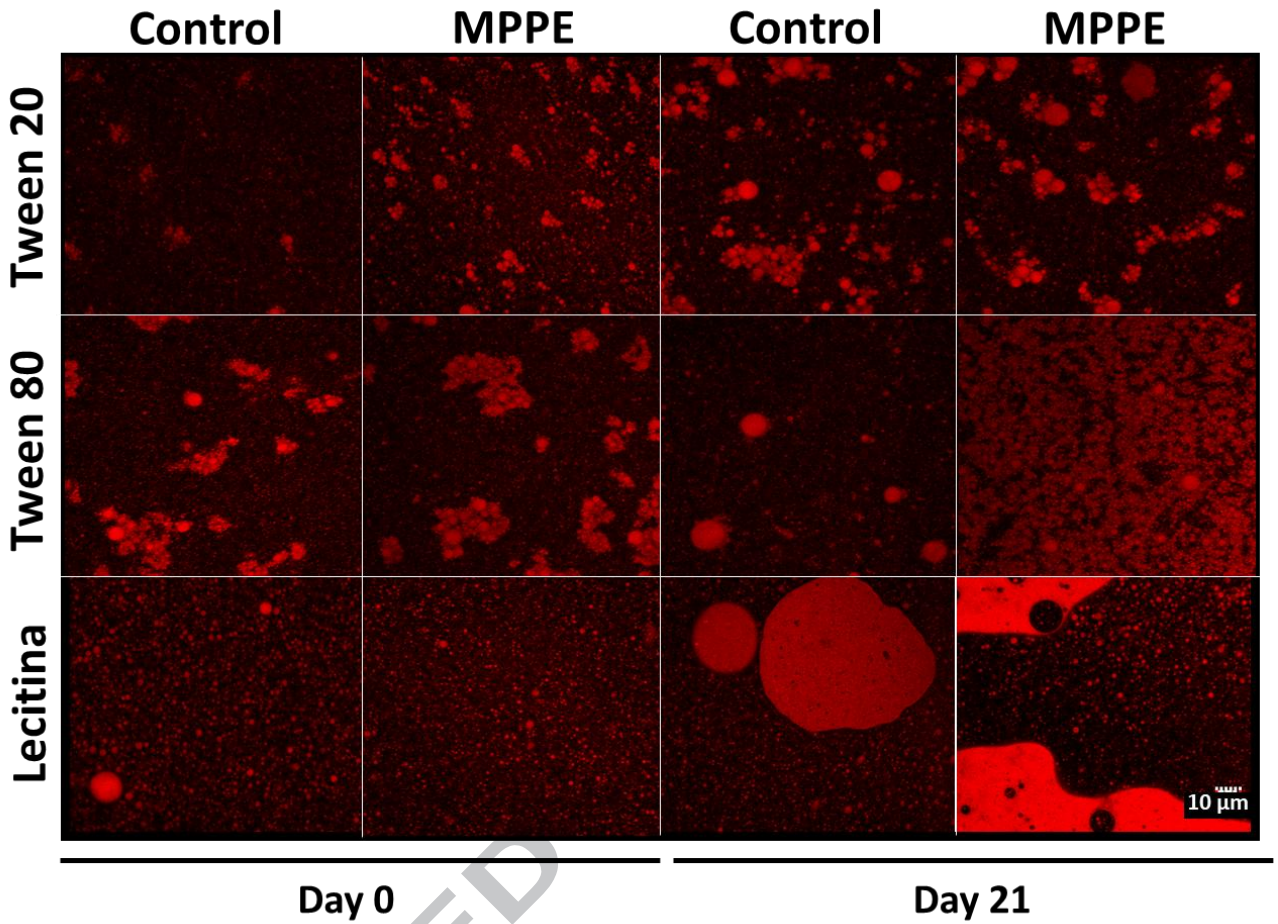

Figure 5. Confocal laser scanning microscopy (CLSM) images of $W_1/O/W_2$ emulsions at 0 and 21 days of storage at 4 °C. MPPE= Mango peel phenolic-rich extract.

TABLES

Table 1. Effect of amplitude and time of ultrasound treatment on particle size and distribution of inner water-in-oil (W_1/O) emulsion.

W_1/O	Amplitude (μm)	Time (min)	$d_{4,3}$ (μm)	SPAN value
Control		1	0.62 ± 0.03 a	1.94 ± 0.18 a
	40	2	0.84 ± 0.51 a	1.64 ± 0.24 ab
		3	0.69 ± 0.03 a	1.81 ± 0.16 b
		1	0.49 ± 0.05 a	1.02 ± 0.48 a
	60	2	0.51 ± 0.07 a	0.90 ± 0.38 a
		3	0.49 ± 0.04 a	1.01 ± 0.53 a
		1	0.51 ± 0.02 a	0.99 ± 0.14 a
	80	2	0.50 ± 0.09 a	1.01 ± 0.38 a
		3	0.38 ± 0.01 b	0.72 ± 0.21 a
		1	0.44 ± 0.01 a	0.86 ± 0.03 a
	100	2	0.38 ± 0.01 b	0.79 ± 0.12 a
		3	0.36 ± 0.01 c	0.65 ± 0.02 b
MPPE		1	0.84 ± 0.27 a	1.87 ± 0.76 a
	40	2	0.56 ± 0.03 b	1.17 ± 0.24 b
		3	0.60 ± 0.01 b	1.11 ± 0.31 b
		1	0.57 ± 0.11 a	1.08 ± 0.43 a
	60	2	0.58 ± 0.11 a	1.06 ± 0.46 a
		3	0.48 ± 0.05 a	1.02 ± 0.49 a
		1	0.68 ± 0.06 a	1.20 ± 0.02 a
	80	2	0.50 ± 0.02 b	0.87 ± 0.21 b
		3	0.39 ± 0.01 c	0.83 ± 0.09 b
		1	0.41 ± 0.01 a	0.81 ± 0.07 a
	100	2	0.40 ± 0.00 a	0.62 ± 0.07 b
		3	0.38 ± 0.00 b	0.54 ± 0.03 c

MPPE = Mango peel phenolic-rich extract. Data are shown as mean ± standard deviation (n=3). Different letters within columns indicate statistically significant differences between time of treatment (minutes) at a single amplitude.

Highlights

- Mango peel phenolic-rich extract (MPPE) was encapsulated using double emulsions.
- Emulsions using Tween 20 and Lecithin had higher encapsulation efficiency (EE).
- Emulsions using Tween 80 had the highest encapsulation stability (ES).
- Control and MPPE emulsions' stability suggest antioxidant-surfactant interactions.
- Confocal laser scanning microscopy (CLSM) revealed flocculation and coalescence.

Graphical Abstract